“ Evaluation of antimicrobial activity of Sulfonamide derivatives”

Master of Science in Biotechnology

Submitted by

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Declaration By The Candidate

I hereby declare that the dissertation entitled "**Evaluation of antimicrobial activity of Sulfonamide derivatives**"submitted by me, for the degree of Master of Science to KIIT University is a record of bona fide work carried by me under the supervision and guidance of **Dr. Debprasad Chattopadhyay;** Scientist G (Senior Deputy Director) of the ICMR Virus Unit, Kolkata and **Dr.chittaranjan sinha ;** professor of chemistry,Jadavpur university, Kolkata.

Date: 05.04.2018

Place: KOLKATA

CONTENT

ABBREVIATION

DHPS : Dihydropteroate synthetase

DNA : Deoxyribonucleic acid

MTT : 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide

NADH: Nicotinamide adenine dinucleotide

PABA : Para-amino benzoic acid

RNA : Ribo Nucleic Acid

Abstract

Sulfonamides, structural analogue of *p*-aminobenzoic acid (PABA), inhibit bacterial folate synthesis. Plant extract is low cytotoxic than that of synthetic compound. In this proposal, molecules have provided (salfathiazole and salfamethoxazole) that carries sulfonamide, synthetic drug, and coumarin,plant extract. Sulfonamide is highly cytotoxic compound, and have examined the cellur cytotoxicity thereof against Vero cells (African green monkey's kidney cells) and check it's antimicrobial activity by broth dilution method and disc diffusion method.

Introduction :-

Antibacterial agent: Antibacterial agents are generally drugs, chemical or other substance that kill or inhibit the growth of bacteria. Bacteriostatic antibiotics limit the growth the bacteria by interfering with bacterial protein production, DNA replication or other aspects of bacterial cellular metabolism. But bactericidal agents totally kill the bacteria. Bacteriostatic agents must work with the immune system to remove the bacteria from the body. High concentrations of some bacteriostatic agents are also bactericidal, whereas low concentrations of some bactericidal agents are bacteriostatic.

Sulfonamide: It is the basis of several groups of drugs. The original antibacterial sulfonamides (sometimes called simply sulfa drug) are synthetic antimicrobial agents that contain the sulfonamide group. In bacteria, antibacterial sulfonamides act as competitive inhibitor of the enzyme dihydropteroate (DHPS), an enzyme involved in folate synthesis. Sulfonamides are structural analog of *p*-amino benzoic acid (PABA), inhibit bacteria folate synthesis. Sulfonamides antagonized by PABA possess an amino group in the **para-**position (labelled C-4) of the sulfone group.[1][2]

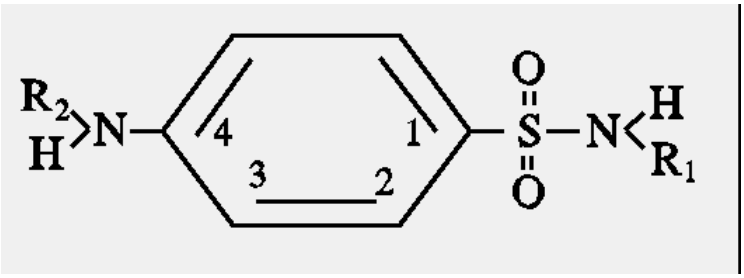
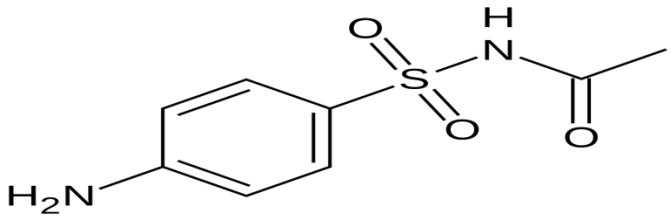
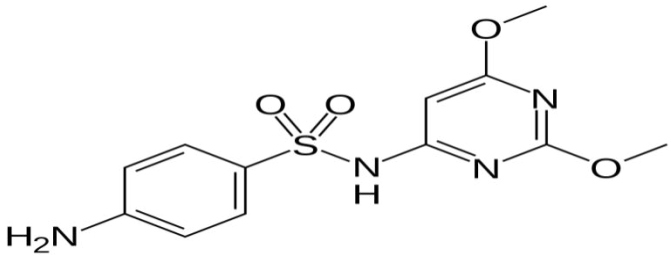
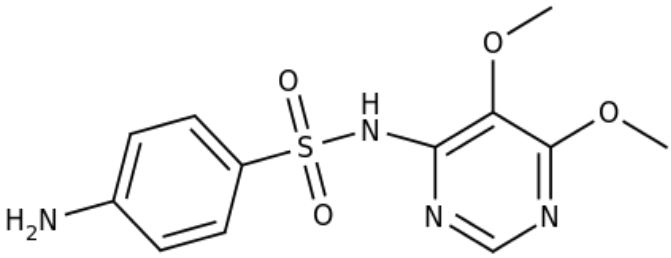
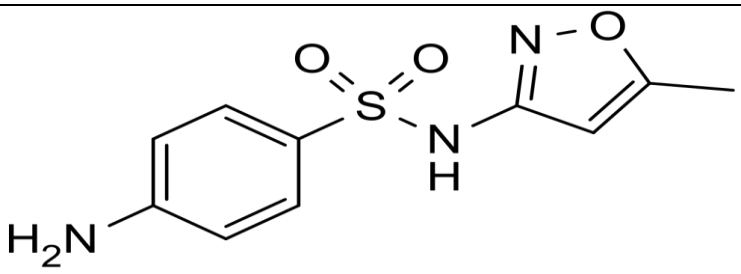
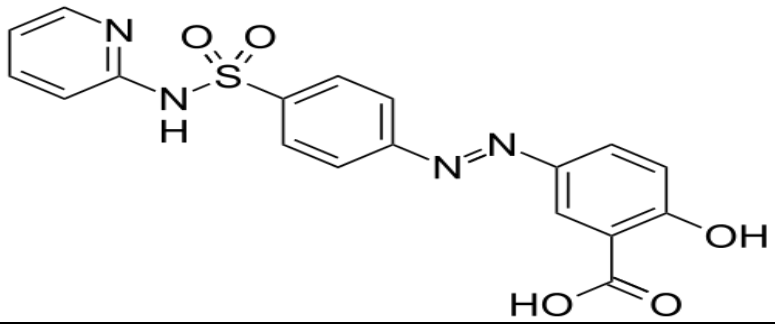
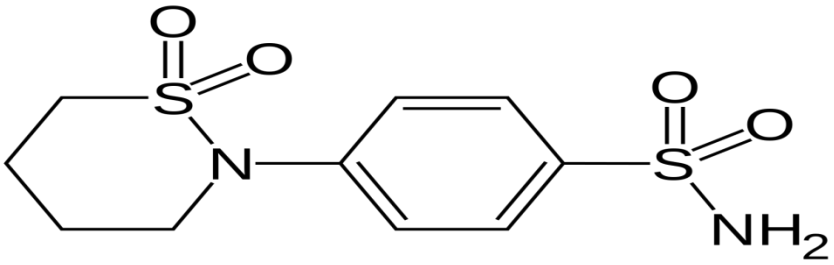


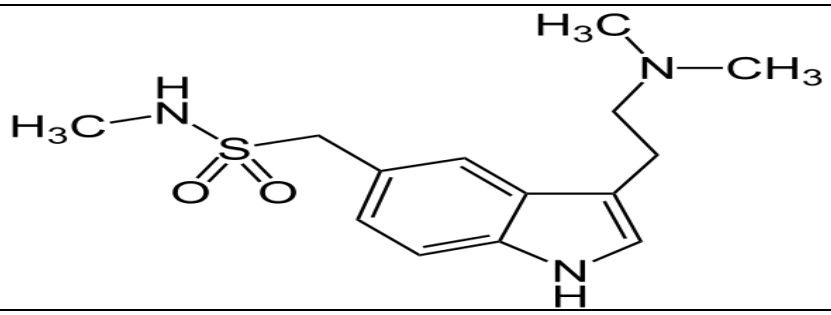
FIG-1 STRUCTURE OF SALFONAMIDE[3].

Sulfonamide Antibiotics (Sulfa Drugs)

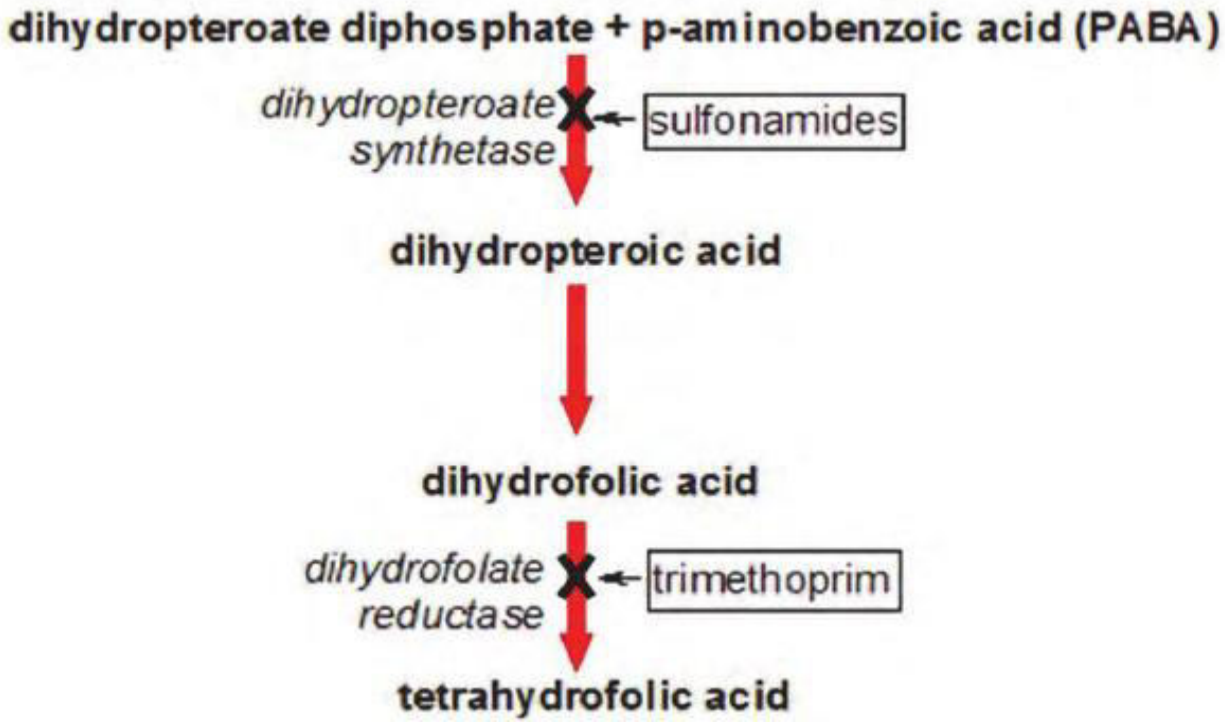
Sulfa drugs are synthetic antimicrobial agents that contain the sulfonamide group. German bacteriologist and pathologist Gerhard Domagk was awarded the 1939 Nobel Prize for Physiology or Medicine for discovering the antibacterial effects of prontosil red, a dye which contained the active component, sulfanilamide.[3]

Some representative sulfonamides include:

Name	Structure
❖ Sulfacetamide	
❖ Sulfadimethoxine	
❖ Sulfadoxine	
❖ Sulfamethoxazole	
❖ Sulfasalazine	
❖ Sultiame	

❖ Sumatriptan	

Mechanism of action of sulfonamide:



Antibacterial sulfonamides is a competitive inhibitors of the enzyme dihydropteroate synthetase, (DHPS). Dihydropteroate synthetase activity is vital in the synthesis of folate, and folate is required for cells to make nucleic acids, such as DNA or RNA.Purine and pyrimidine production are stopped by the inhibition of folic acid synthesis cycle.For this reason replication process can not occur in bacterial cell . So if DNA molecules cannot be built, the cell cannot divide, and the effect is bacteriostatic (rather than bactericidal).

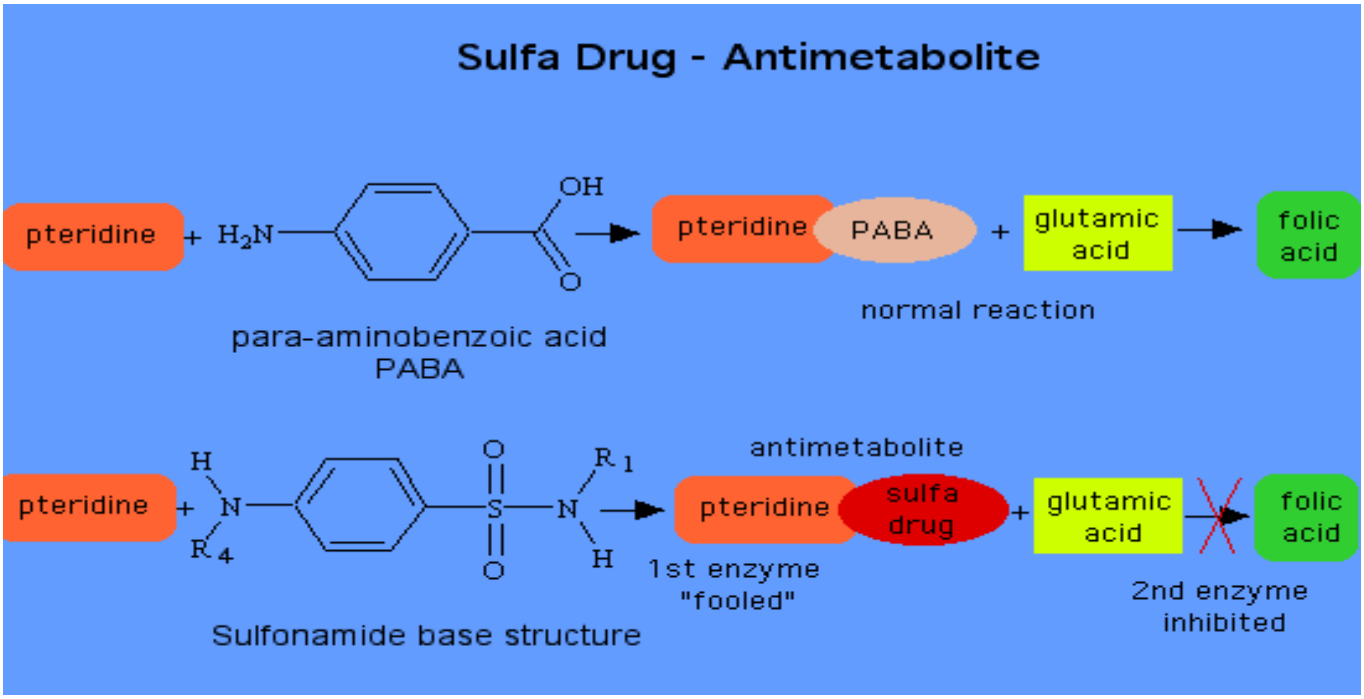


FIG-2 SULFA DRUG-ANTIMETABOLITE(TAKEN FROM C.OPHARDT.C.2003)

Normally folic acid is synthesized in two steps in bacteria by the above reaction . If sulfa drug is used, the first enzyme is not to specific and can use the sulfonamide in the first reaction. This reaction produces the product containing pteridine and the sulfa drug. The next and final step is the reaction PABA + with glutamic acid to make folic acid. If the sulfa drug has been substituted for the PABA, then the final enzyme is inhibited and no f

olic acid is produced[4][5][6].

Antimicrobial Spectrum of Sulfa Drugs:

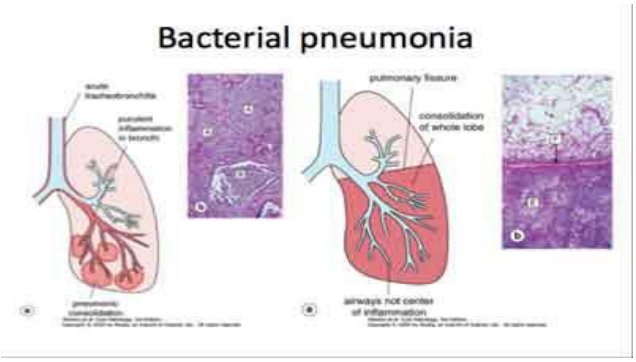
Sulfonamides have broad spectrum activity against both

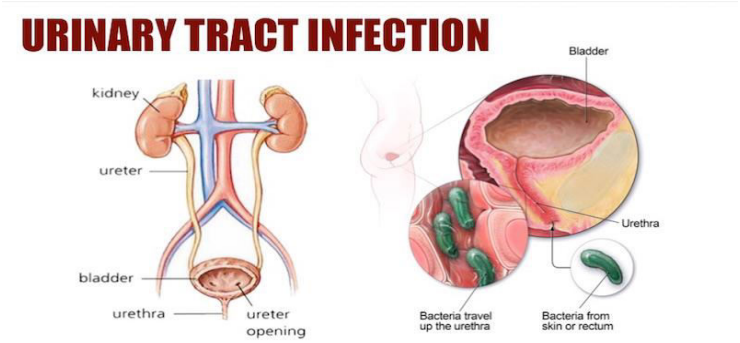
A. Gram-positive bacteria and

B.Gram-negative bacteria.

Type of Infections Sulfa Antimicrobic Drugs are Used Against:

Sulfa drugs are used to treat some types of bacterial pneumonia, urinary tract infections, shigellosis, *Nocardia* infections (a type of bacterium with an unusual waxy cell wall) and specific protozoal infections.





Allergies to sulfonamides are common (about 3% of general population), so sulfa drugs are prescribed carefully. Hypersensitivity reactions are less frequently seen in non-antibiotic sulfonamides. The most common hypersensitivity reaction to sulfa drugs is skin reactions such as rashes and hives. However, there are several types of reactions that can be life-threatening [4-6].

Toxicity of Sulfonamides:

- **Hypersensitivity:** allergic reactions including skin rashes and fever. Cross allergy may occur with chemically related drugs (thiazides, hypoglycemics)

- **GI:** nausea, vomiting and diarrhea

- **Hematotoxicity:** they are rare. Granulocytopenia, thrombocytopenia and aplastic anemia

- **Nephrotoxicity:** they may precipitate in the urine at acidic pH, causing crystalluria and hematuria

REVIEW OF LITERATURE

Sulfonamide exhibit broad range of biological activities. Several sulfonamides are used in therapy such as celecoxib, nimesulide, delavirdine, acetazolamide, furosemide. So far modifications of the sulfonamides have proven highly effective and modifications that have been made so far do not exhaust the possible changes that can be made to improve potency and efficacy of these sulfonamides[7].

The term sulfonamide is commonly used to refer to antibacterials that are

(i) Aniline substituted sulfonamides, the sulfonamides, (ii) prodrugs that produce sulfonamide ,(iii) nonaniline sulfonamides[7].

These studies led to discovery of many useful therapeutically active drugs namely furosemide (high ceiling diuretic) ,thiazide derivatives (diuretic agents), sulfonylureas (antidiabetic agents), sotalol (Beta-blocker) and delavirdine (anti HSV agent).

Antimicrobial activity

The antibacterial action of sulfonamides is by way of interference with bacterial biochemical reaction lacking in man and represents the first magic bullet. The discovery of antimetabolite mechanism of action led to the synthesis of analoguses of metabolite p-aminobenzoic acid and (PABA)[8] (Fig-1) and other sulfonamide derivatives. But efforts for synthesis of analogues PABA were not successful in synthesizing useful compounds. Bharmal et al[9] synthesized some new N-aryl-sulfonamido-2-chloro-8-methylquinolin-3-yl-azo-methine (Fig-2) and tested them for antimicrobial activity against gram positive and gram negative bacterial strains and antifungal activity towards *Aspergillus niger*. The compounds were moderately active against different strains of bacteria and fungus. Significant activity was observed in antimicrobial having R = 4-acetamidophenyl, 3-carboxy-4-chlorophenyl,3-carboxy-6-methylphenyl and 2,4-dichlorophenyl Shah et al.[10] synthesized some new sulfonamide arylamides and thiourea derivatives having 2-amino-4(6'-methoxy--naph-thyl)-thiazole moiety. The compounds were screened for antimicrobial activity against *B.megate, B.subtilis, E.coli, P.fluorescence and A. awamori*. Most of the compounds showed mild to moderate antimicrobial activity. Patel et al.[11] reported the synthesis and antimicrobial of sulfonamide derivatives (Fig-3).The compounds containing R=4-methylphenyl showed moderate to good antimicrobial activity that was comparable with standard drug ampicillin against *B.subtilis*, R=3-carboxy-4-mefhylphenyl showed chowed comparable activity with standard drug norfloxacin against *E.coli*. Joshi et al.[12] carried out synthesis and comparative study on antibacterial activity of sulfonamides derived from them antibacterial activity against various gram negative bacteria and were analyzed statistically .The result have shown that the compounds are quit active against pathogen under study and were nontoxic.

Anti HIV activity: Lysinesulfonamide and its structural analogs, a class of Human Immunodeficiency Virus protease inhibitors has gained importance in recent years due to its mode of action. QSAR analysis for multiple ligand-receptor complexes can be performed using binding interaction energies derived from the molecular dynamics simulations. A Receptor-dependent QSAR (RD-QSAR) analysis was carried out for 65 lysine sulfonamide analogs complexed with HIV-protease using Prime Molecular Mechanics Generalized Born Surface Area (MM-GBSA) method. The lysine sulfonamide analogs were docked in the receptor active site and the obtained complexes were further rescored using Prime MM-GBSA method. The descriptors, docking score and MM-GBSA free energy of binding were used to derive a relationship with biological inhibition constant. The influence of individual free energy components on biological activity and the effect of structural flexibility (in terms of strain energies) over the prediction model has been studied using two models (with and without strain energy), built using forward entry MLR method. Inclusion of strain energies enhanced the effect of all the free energy of binding components and hence reflects their importance. The statistical significance of the derived QSAR models was described using the parameters, F-test and RMSE. A test set of 11 compounds was used to ensure the predictability of the models (PRESS). Results from this study would be useful in identifying anti-viral with energetically favorable interactions[13].

4-[(1,2-Dihydro-2-oxo-3H-indol-3-ylidene)amino]-N(4,6-dimethyl-2-pyrimidinyl)-benzene sulphonamide and its derivatives were synthesized by reaction and its derivatives with sulphadimidine. Their chemical structures have been confirmed by IR, (1)H NMR data and elemental analysis. Investigation of anti-HIV activity of compounds were tested against replication of HIV-1 (IIIB) and HIV-2 (ROD) strains in acutely infected MT-4 cells and the activity compared with standard azidothymidine. Among the compounds tested, 4-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)amino]-N(4,6-dimethyl-2-pyrimidinyl)-benzene sulphonamide and its N-acetyl derivative were the most active compounds [14].

Plant extract is less cytotoxic than the synthetic compound. sulfonamide is synthetic compound.Sulfonamide is highly cytotoxic compound. schiff base (sulfonamide was attached with coumarin aldehyde) has low cytotoxic power because Schiff is made by plant extract and synthetic compound.

Anticancer activity : The sulfonamides constitute an important class of drugs, with several types of pharmacological agents possessing antibacterial, anti- carbonic anhydrase, diuretic, hypoglycemic and antithyroid activity among others. A large number of structurally novel sulfonamide derivatives have ultimately been reported to show substantial antitumor activity in vitro and in vivo. Although they have a common chemical motif of aromatic/heterocyclic or amino acid sulfonamide, there are a variety of mechanisms of their antitumor action, such as carbonic anhydrase inhibition, cell cycle perturbation in the G1 phase, disruption of microtubule assembly, functional suppression of the transcriptional activator NF-Y, and angiogenesis (matrix

metalloproteinase, MMP) inhibition among others. Some of these compounds selected via elaborate preclinical screenings or obtained through computer-based drug design, are currently being evaluated in clinical trials. The review summarizes recent classes of sulfonamides and related sulfonyl derivatives disclosed as effective tumor cell growth inhibitors, or for the treatment of different types of cancer. Another research line that progressed much in the last time regards different sulfonamides with remarkable antiviral activity. Thus, at least two clinically used HIV protease inhibitors possess sulfonamide moieties in their molecules, whereas a very large number of other derivatives are constantly being synthesized and evaluated in order to obtain compounds with less toxicity or activity against drug-resistant viruses. Several non nucleoside HIV reverse transcriptase or HIV integrase inhibitors containing sulfonamido groups were also reported. Another approach to inhibit the growth of retroviruses, including HIV, targets the ejection of zinc ions from critical zinc finger viral proteins, which has as a consequence the inhibition of viral replication in the absence of mutations leading to drug resistance phenotypes. Most compounds with antiviral activity possessing this mechanism of action incorporate in their molecules primary sulfonamide groups. Some small molecule chemokine antagonists acting as HIV entry inhibitors also possess sulfonamide functionalities in their scaffold.[15]

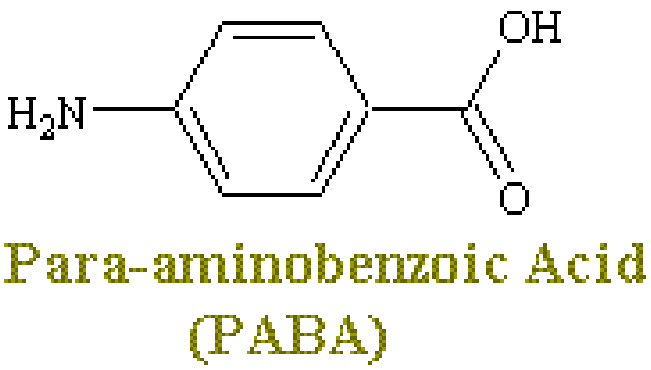
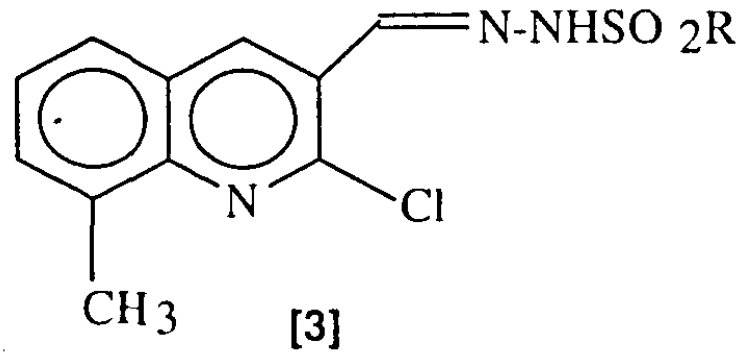


Fig-1



[3]

Fig-2

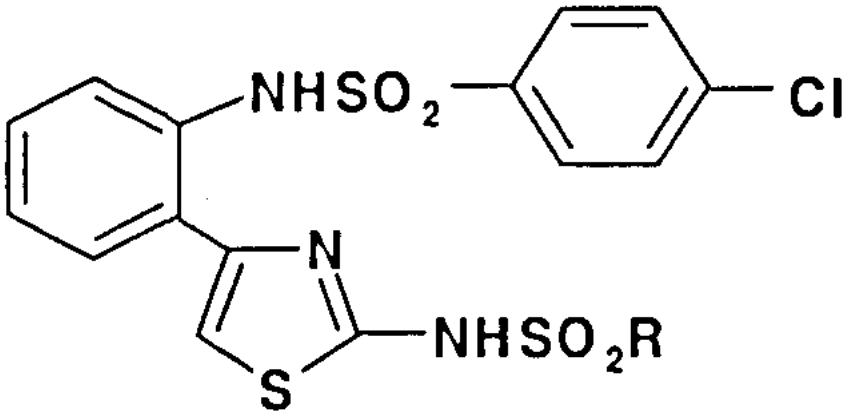


Fig-3

OBJECTIVE:

Sulfonamides are structural analog of *p*-amino benzoic acid (PABA), inhibit bacteria folate synthesis. Sulfonamides antagonized by PABA possess an amino group in the fourposition of the sulfone group. Here, we want to chemically modify sulfonamide for lowering its cytotoxicity without hampering its activity.

The antimicrobial activity of all the synthesized compounds will be examined against Gram-positive bacteria (*Staphylococcus aureus)* and Gram-negative (*Escherichia coli*).

1. Different types of Sulfonamide's derivatives will be taken.

2. Antibacterial activity will be measured by following methods:

A. Zone of inhibition.

B. Broth dilution method.

3. MTT assay for cytotoxicity.

MATERIALS & METHODOLOGY:

Minimal inhibitory concentration (MIC)

Culture media:

1. Muller Hinton Agar (MHA, Merck)
2. Sterile normal saline solution (NSS)
3. Heart infusion broth (HIB, Difco)
4. Sodium chloride (NaCl)

Solvents:

1. Distilled water
2. 0.1 N sodium hydroxide (NaOH)
3. Absolute ethanol
4. Dimethyl sulfoxide
5. N-N-Dimethyl formamide

Culture :

1. Gram-positive bacteria.***(Staphylococcus aureus)***
2. Gram-negative bacteria.***(Escherichia coli)***

Composition and preparation of culture media and reagents

Mueller Hinton II agar[16]

Ingredients Gms / Litre
Beef extract 2.0 g
Acid hydrolysate of casein 17.5 g
Starch 1.5 g
Agar 17.0 g
Distilled water 1000 ml

Preparation:
Dissolve the dehydrated medium in water by heating if necessary. Adjust pH to 7.2 - 7.4, transfer into bottles and autoclave at 110 ^{o}C for 20 min.

Nutrient Agar[17][18]

Ingredients Gms / Litre
Peptic digest of animal tissue5.000
Sodium chloride 5.000
Beef extract 1.500
Yeast extract 1.500
Agar 15.000

Final pH (at 25°C) 7.4±0.2

Saline solution

Ingredients Gms / Litre
Sodium chloride 8.5 g
Water 1000 ml

Preparation:
Dissolve the sodium chloride in the water by heating if necessary. Adjust pH to 7.0 after sterilisation. Dispense the 4 ml solution into tubes after autoclaving at 121 ^{o}C for 20 min.

Nutrient Broth Medium[19][20][21]

Ingredients Gms / Litre
Peptone 10.000
Beef extract 10.000
Sodium chloride5.000
pH after sterilization 7.3±0.1

Procedure

Broth dilution is a method used to test the susceptibility of bacteria to antibiotics.

Take 9 sterilized test tube and add 2ml media for each tube,and add culture equal amount in each test tube.

Test tubes is keeping for overnight incubation.

Then add antibiotics according to low to high concentration (100ul, 200ul,250ul-600ul) except one this is for stock and incubate it for 12 hours.

Then take OD at 610nm

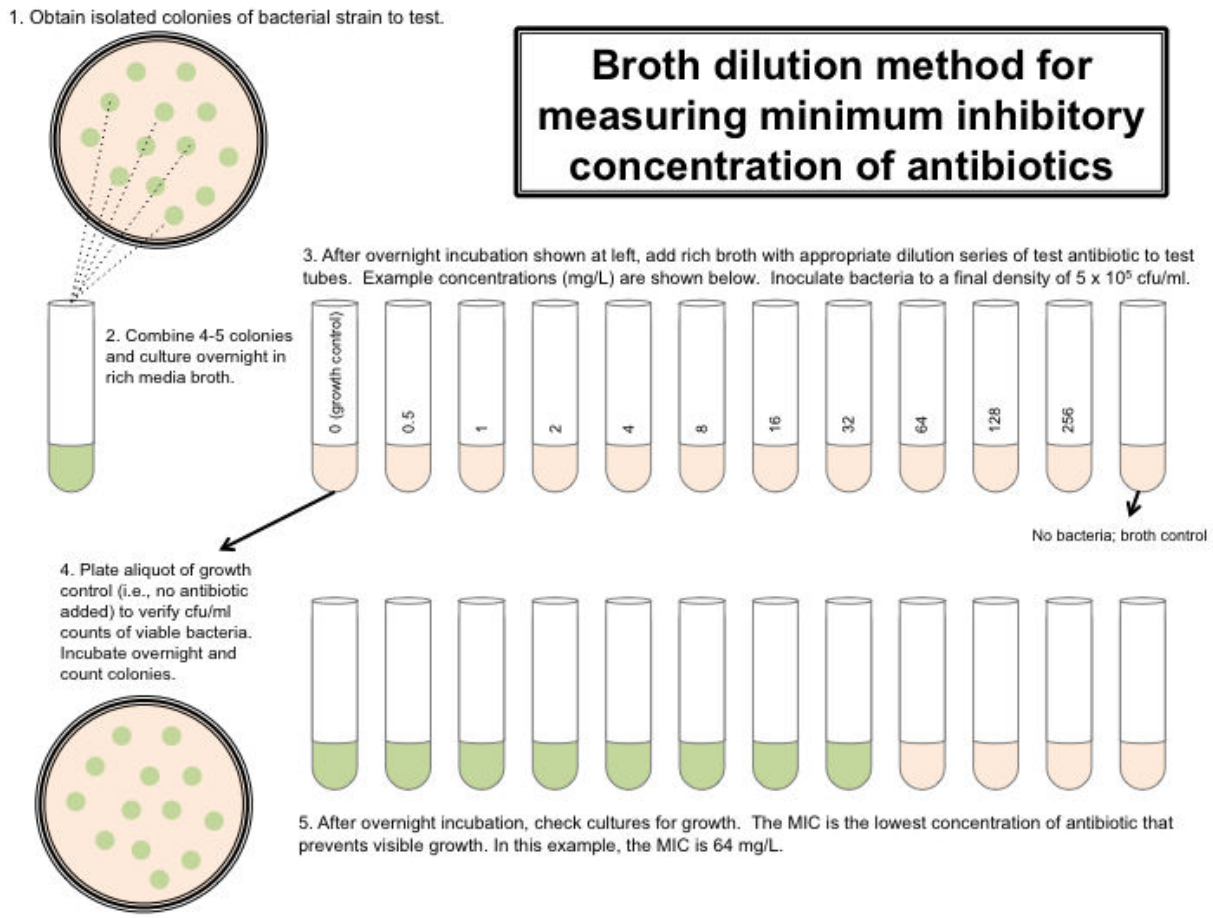


FIG- 3 :Broth dilution method for measuring minimum inhibitory concentration of antibiotics

Antibiotic Sensitivity testing: The Kirby-Bauer Disc Diffusion Test:

Certain bacteria can display resistance to one or two or more antibiotics. Determining bacterial **antibioticresistance** – whether a bacterium can survive in the presence of an antibiotic-is a critically important part of the management of infectious diseases in patients. The **Kirby-Bauer (K-B) disk diffusion test** is the most common method for antibiotic resistance/susceptibility testing. The results of such testing help physicians in choosing which antibiotics to use when treating a sick patient.The Kirby-Bauer (K-B) test utilizes small filter disks impregnated with a known concentration ofantibiotic. The disks are placed on a Mueller-Hinton agar plate that is inoculated with the test microorganism. Upon incubation, antibiotic diffuses from the disk into the surrounding agar. If susceptible to the antibiotic, the test organism will be unable to grow in the area immediately surrounding the disk, displaying a **zone of inhibition**. The size of this zone isdependent on a number of factors, including the sensitivity of the microbe to the antibiotic, the rateof diffusion of the antibiotic through the agar, and the depth of the agar. Microorganisms that are resistant to an antibiotic will not show a zone of inhibition or display a relatively small zone.Here testing a number of bacteria against various antibiotics. Following inoculation and incubation we will assess the results by observing whether any zones of inhibition are formed, accaoding their sizes, and comparing our results with those obtained.

Materials needed:

- □ Test tube rack
- □ Bacterial incinerator
- □ Forceps
- □ Sterile swabs
- □ Two Mueller-Hinton agar plates

□ Antibiotic disks – each pair of students will be using:

(i) **TWO** different antibiotics

(ii) Antibiotic-free disks (BLANK)

□ Stock broth cultures of:

- *Escherichia coli* (Gram negative)

- *Staphylococcus aureus* (Gram positive)

Record which antibioticsare using here:

1. sulfathiazole

2. sulfamethoxazole

Procedure:

Label the agar plates . Also mark, using dots, where i will put the antibiotic disks and the STANDARD (Gentamicine) disk. Discs is a minimum of 20 mm apart. Inoculate one plate with my first bacterium. Using aseptic technique, wet a swab with the bacterial broth culture. Thoroughly swab the surface of the plate, making sure to cover the entire surface.100ul culture. Then make spread plate.Sterilize the spreader every times. Place one antibiotic disk onto the surface of the agar, using aseptic technique.Heat the tips of the forceps by placing them just inside the opening for 5-10 seconds. Cool the forceps by waving them in the air for about 10 seconds. Carefully pick up your test disk with the forceps, and gently place it in the appropriate spot on the agar surface,To ensure that the disk is flat on the agar, gently push it down with the forceps. Reheat the tips of the forceps as above to kill any bacteria. Repeat the procedure with the second antibiotic disk.Repeat the procedure with the STANDERED disk.Repeat steps on a new agar plate with your second bacterium. Wait until the surface of the plates has completely dried. Incubate both plates at **37°C**.

1.Take sterilized plate and add Mueller Hinton agar and cool it

2.Add 100ul culture and make spread plate

3.Incubate for 12 hours at 37'C

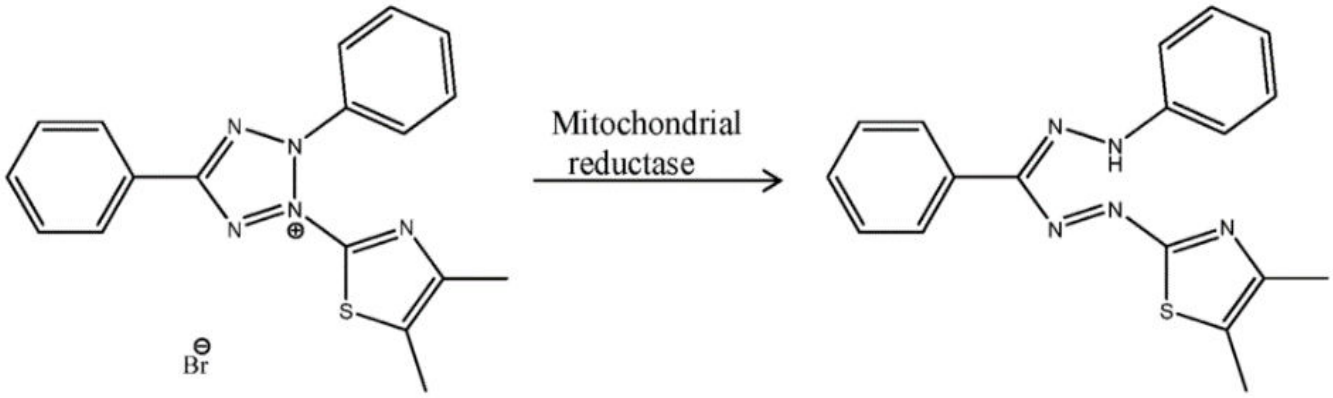
4.Add stander antibiotic tablet (Gentamicin) and another antibiotic in different concentration (100,200,300ug/ml)

5.Incubate at 37'C for 12 hours

MTT assay:

Measurement of cell viability and proliferation forms the basis for numerous *in vitro* assays of a cell population's response to external factors. The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation. The yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means. The MTT Cell Proliferation Assay measures the cell proliferation rate and conversely,when metabolic events lead to apoptosis or necrosis, the

reduction in cell viability. The number of assay steps has been minimized as much as possible to expedite sample processing. The MTT Reagent yields low background absorbance values in the absence of cells. For each cell type the linear relationship between cell number and signal produced is established,thus allowing an



accurate quantification of changes in the rate of cell proliferation.

FIG- 4. MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide)

Materials and methods:

Cell and viruses: Vero cells (African green monkey kidney cells; ATCC, Manassas, VA, USA) were cultured in Dulbecco's modified Eagle's medium (DMEM) with 5–10% fetal bovine serum (FBS; Invitrogen, USA), 100 U ml^{-1} penicillin and 100 mg ml^{-1} streptomycin, at 37 ^{0}C in 5%CO2. The viral strains used were HSV-1F (ATCC 734), purchased from the ATCC (Manassas, VA, USA). Virus stocks were prepared from infected culture at a multiplicity of infection (moi) of 0.5 for 1 h at 37^{0}C. The residual viruses were then washed out with phosphate-buffered saline (PBS) and the cells were cultured for another 48–72 h. The cultured cells were lysed finally by three cycles of freezing and thawing, centrifuged at 1500g at 4^{0}C for 20 min and the collected supernatant was tittered by plaque assay, and stored at -80^{0}C for further studies.

Biological evaluation:

Determination of cytotoxicity by MTT assay

The effect of compounds on African green monkey kidney cells Vero cell morphology was determined by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay following the manufacturer's instruction (MTT 2003,Sigma-Aldrich, MO)[23].Vero cells were cultured onto 96-well plates at 10×10^6 cells per well, and different concentrations of the compounds(7,7c,7e,7f and7g) were added to each well at a final volume of 100 µl, in triplicate using DMSO (0.1%) and acyclovir (0–50 µg ml^{-1}) as a negative and positive control, respectively. The drug-treated cells were incubated at 37°C with 5% CO2 for 2 days, and then the MTT reagent (10 µl) was added to each well. After 4 h of incubation, the formazan was solubilized by adding MTT solubilisation solution equal to the original culture media volume, and the absorbance was read at 570 nm with a reference wavelength of 690 nm by an ELISA reader. Data were calculated as the percentage of cell viability by the formula: [(sample absorbancecell free sample blank)/mean media control absorbance)]/100%. The 50% cytotoxic concentration (CC_{50}) causing visible morphological changes in 50% of Vero cells with respect to cell control was determined (Zhang et al. 2007; Bag et al. 2012; Mukherjee et al. 2013)[24].

Result:

Minimal inhibitory concentration (MIC) of sulfonamide on E.coli

The O.D of each concentration of antibiotic was measured by Spectrophotometer

Sulfathiazole (E.coli)

Concentration	OD
100µg/ml	0.217
150µg/ml	0.155
200µg/ml	0.135
250µg/ml	0.1285
300µg/ml	0.119
400µg/ml	0.107
500µg/ml	0.092
600µg/ml	0.012
Bac *E.coli*	0.698

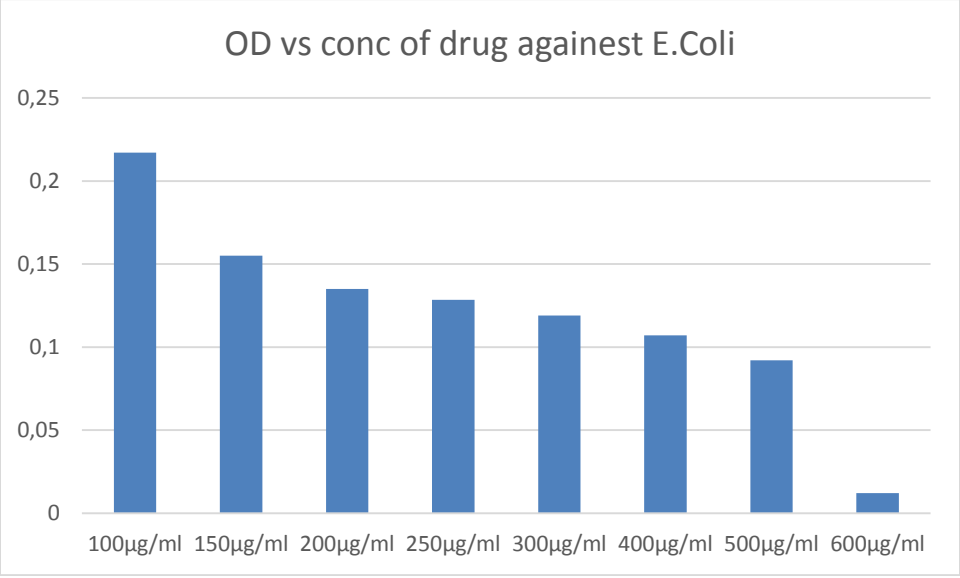


FIG- 6 : OD Vs Conc of drug againest *E.coli*

Sulfathiazole (***Staphylococcus aureus***)

Concentration	OD
100µg/ml	0.523
150µg/ml	0.3275
200µg/ml	0.275
250µg/ml	0.2535
300µg/ml	0.2395
400µg/ml	0.2035
500µg/ml	0.177
600µg/ml	0.129
Bac SA	0.61

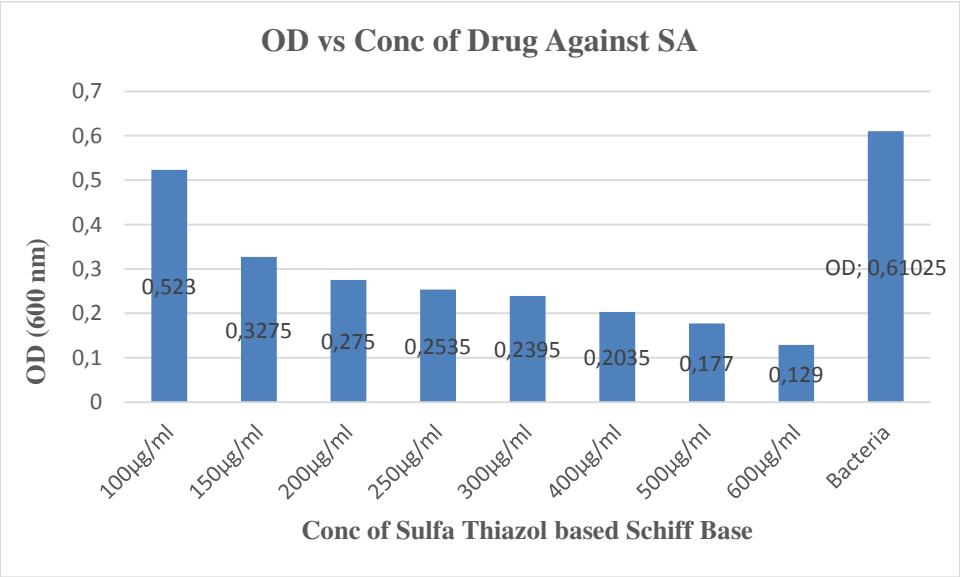


FIG-7 ; OD vs Cons of drug against *Staphylococcus aureus*

Sulfamethoxazole (***Staphylococcus aureus***)

Concentration	OD
100µg/ml	0.179
150µg/ml	0.176
200µg/ml	0.1715
250µg/ml	0.152
300µg/ml	0.144
400µg/ml	0.133
500µg/ml	0.049
600µg/ml	0.2585
Bac SA	0.61

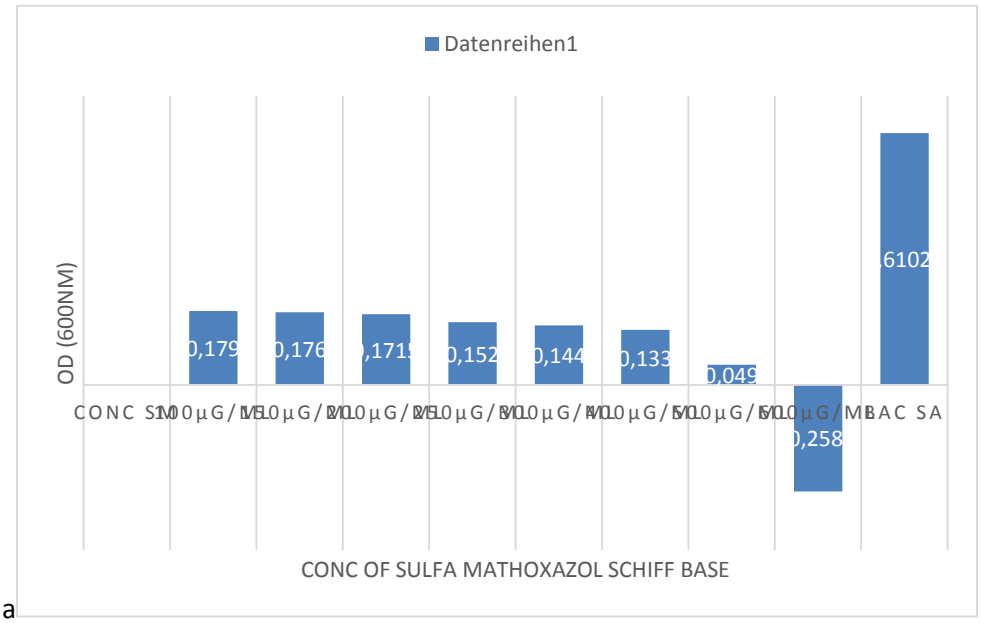


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FIg : OD vs Cons of drug against *Staphylococcus aureus*

Sulfamethoxazole (***Escherichia coli)***

Concentration	OD
100µg/ml	0.577
150µg/ml	0.3325
200µg/ml	0.2285
250µg/ml	0.2175
300µg/ml	0.2055
400µg/ml	0.2005
500µg/ml	0.1435
600µg/ml	0.295
Bac SA	0.713

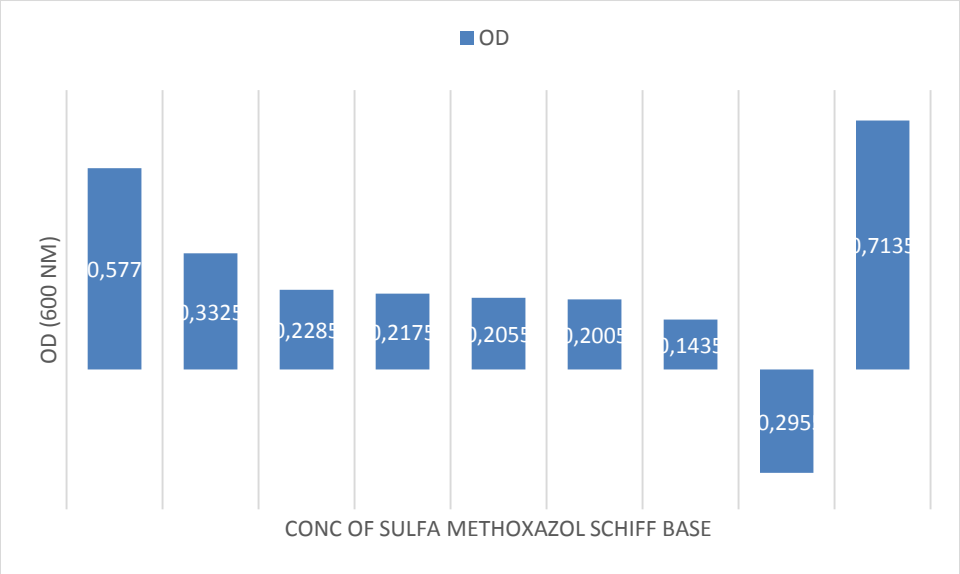


Fig : OD vs Cons of drug against *E.Coli*

The graph was plotted concentration of the antibiotic was taken in increasing order on X asix.The OD was taken on Y axis.

In concentration OD graph a descending curve was obtain.with increase in the concentration antibiotic,the OD value decrease as the bacterial growth was hindered while with the increasing concentration of antibiotics as the given organism was sensitive to high antibiotic concentration.

Antibiotic Sensitivity testing: The Kirby-Bauer Disc Diffusion Test

After 24 hour incubation

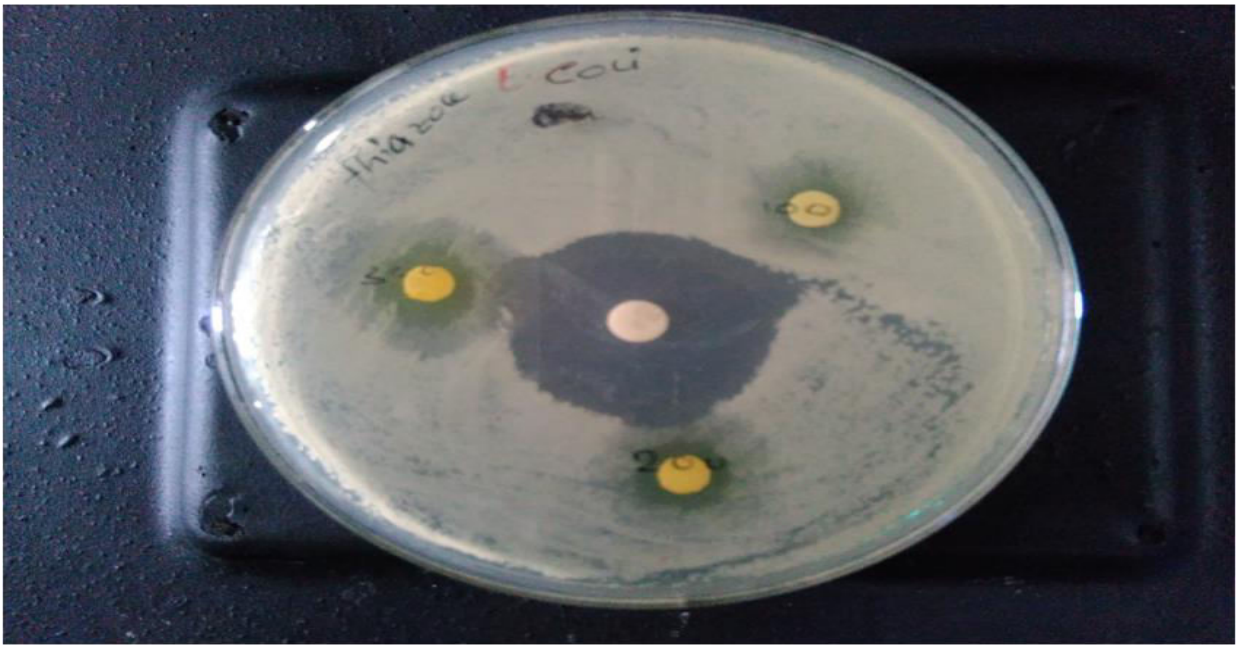
1. Observe both agar plates.

2. If present, measure the diameter of the zone of inhibition in mm.

3. We will then compare the results for the entire class.

Sizes of zones of inhibition (in mm)

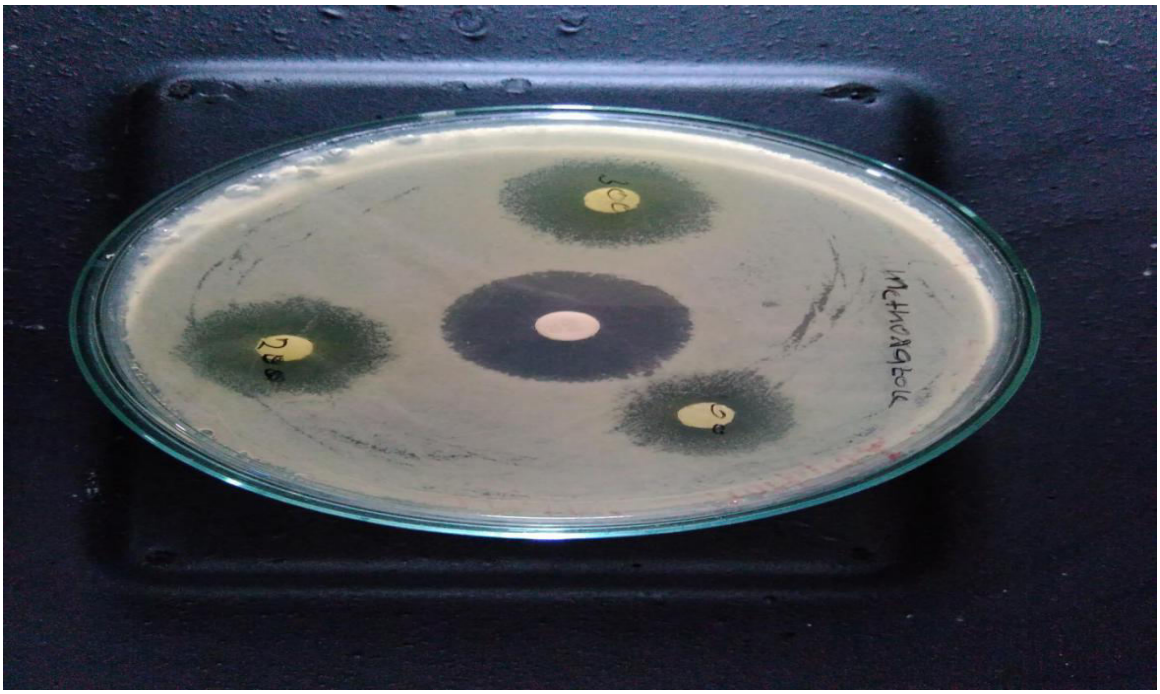
1. sulfathiazole ***(Escherichia coli)***



Disc content (ug/ml)	Zone (mm)
100	17 18 17
200	19 20

	19
300	21 20 20

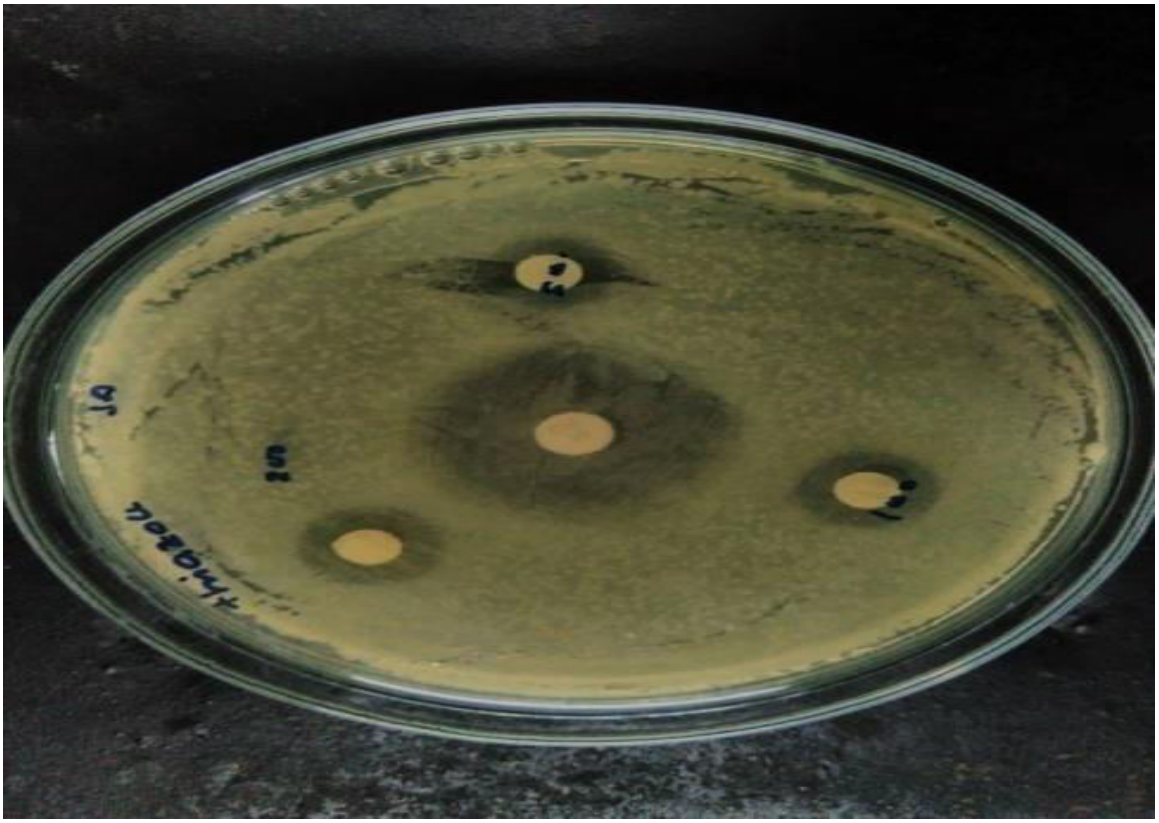
2. sulfamethoxazole ***(Escherichia coli)***



Disc content (ug/ml)	Zone (mm)
100	19 18 19
200	20 20 21

300	21 20 21

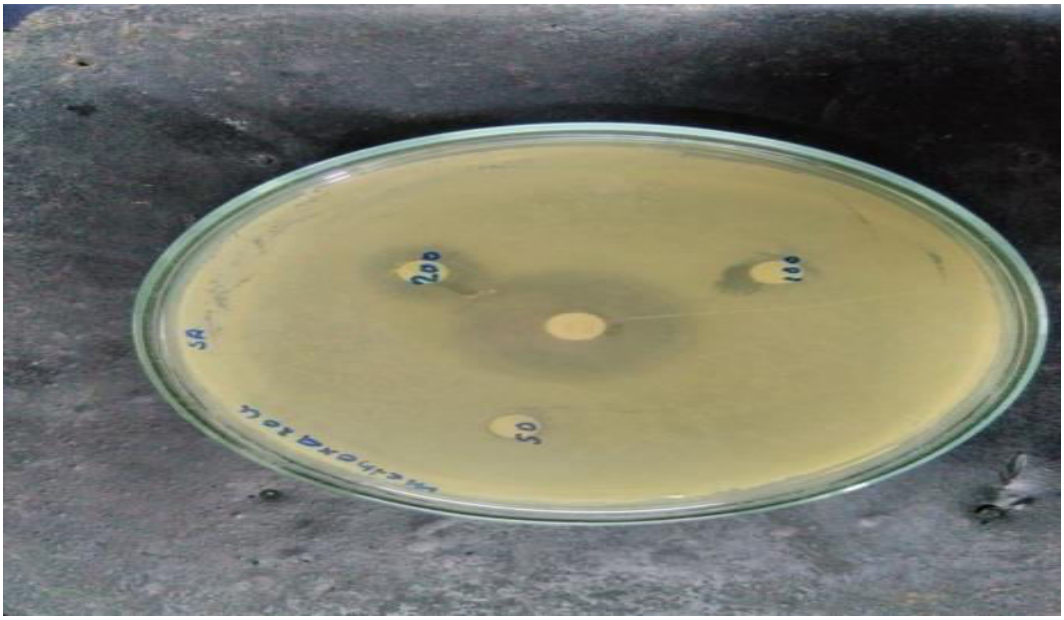
1. sulfathiazole .***(Staphylococcus aureus)***



Disc content (ug/ml)	Zone (mm)
50	8 8 7
100	15 16 15
150	20

	20 21

2. sulfamethoxazole .***(Staphylococcus aureus)***



Disc content (ug/ml)	Zone (mm)
50	NIL
100	15 15 17
150	19 20 20

Zone of inhibition around sulfathiazole and sulfamethoxazole (derivatives of sulfonamides) indicate that the provided microorganism was not resistant against sulfathiazole and sulfamethoxazole.

MTT assay :

The cytotoxicity assay of the compounds indicated that 50% cytotoxicity (CC50) of the compounds Sulfathiazole and sulfamethoxazole were 50,100,150,200,250,300,400 µg/ml respectively.We then determined the cytotoxic effects of each compounds.And the percent of cell viability values are 91,72.16,69.31,62.56,40.04,26.98,17.13.

Sulfathiazole

Conc	
50µg/ml	91
100µg/ml	72.16
150µg/ml	69.31
200µg/ml	62.56
250µg/ml	40.04
300µg/ml	26.98
400µg/ml	17.13

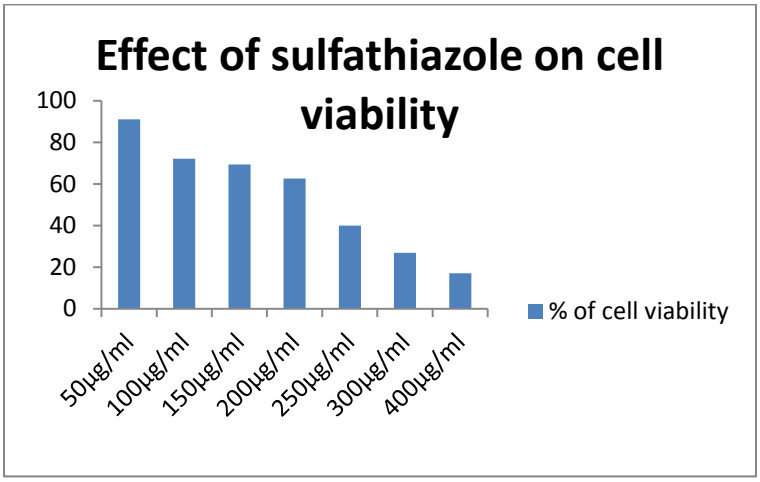


FIG- 8 : Effect of sulfathiazole on cell viability

Sulfamethoxazole

Conc	
50µg/ml	89
100µg/ml	73.16
150µg/ml	71
200µg/ml	60.5
250µg/ml	35.75
300µg/ml	27.39
400µg/ml	19.59

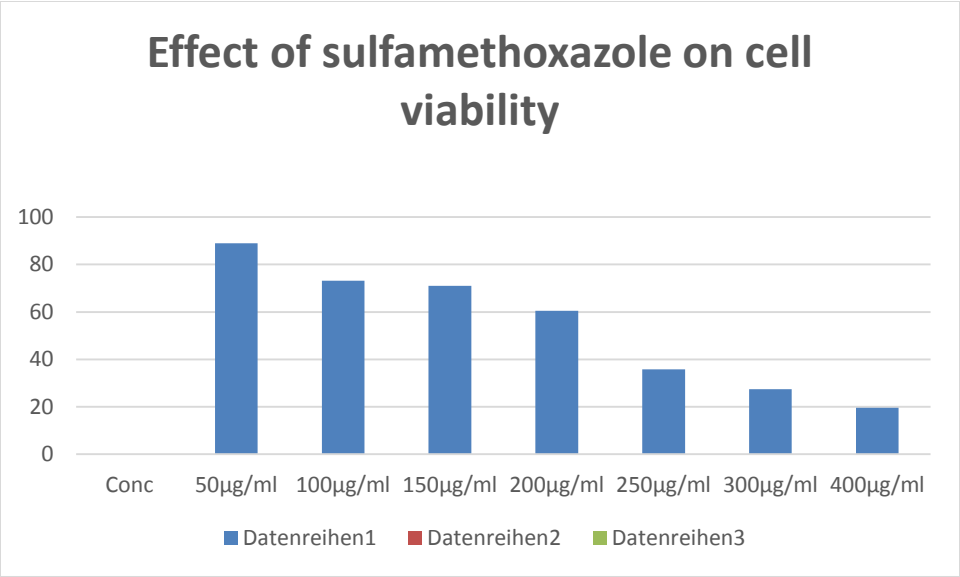


Fig : Effect of Sulfamethoxazole on cell viability

Discussion:

Zone of inhibition around sulfathiazole and sulfamethoxazole (derivatives of sulfonamides) indicate that the provided microorganism susceptible against sulfathiazole and sulfamethoxazole.sulfathiazole and sulfamethoxazole are showing antibacterial activity at 100,200 and 300 ug/ml concentration. plant extract is less cytotoxic than the synthetic compound. sulfonamide is synthetic compound.Sulfonamide is highly cytotoxic compound. schiff base (sulfonamide was attached with coumarin aldehyde) has low cytotoxic power because Schiff is made by plant extract and synthetic compound. After checking MTT assay, we can conclude that sulfonamide's cytotoxicity is higher than the Schiff base cytotoxicity.

Conclusion

1.Sulfathiazole and sulfamethoxazole are showing antibacterial activity at 100,200 and 300 ug/ml concentration.

2.Plant extract is less cytotoxic than the synthetic compound. sulfonamide is synthetic compound.Sulfonamide is highly cytotoxic compound. schiff base (sulfonamide was attached with coumarin aldehyde) has low cytotoxic power because Schiff is made by plant extract and synthetic compound.

3. After checking MTT assay, we can conclude that sulfonamide's cytotoxicity is higher than the Schiff base cytotoxicity.

4. Sulfathiazole is less cytotoxic than the sulfamethoxazole.

Future Work

For these molecules, the desirable action has a high toxicity, so constituting a promising hit for further structure optimization and development of potential antimicrobial agents. Further studies are required to deeply understand of their properties

Reference:

1. Henry R.J. *"The Mode of Action of Sulfonamides"*. Bacteriological reviews.(1943) 7 (4): 175–262. *PMC 440870* . *PMID 16350088*

2. "SULFONAMIDE CLASS ANTIBIOTICS". chemicalland21.com. Retrieved 17 January 2014.

3. American Chemical Society (2000). The Pharmaceutical Century: Ten Decades of Drug Discovery. Washington, DC: ACS. Williams, David A., and Lemke, Thomas L. (2002). Foye's Principles of Medicinal Chemistry, 5th edition. Baltimore: Lippincott Williams & Wilkins. Wolff, Manfred E., ed. (2003). Burger's Medicinal Chemistry and Drug Discovery, 6th edition. New York: Wiley.

4. Bauman, R. Microbiology with Diseases by Taxonomy. Pearson Benjamin Cummings(2014).
5. Park Talaro, K. Foundations in Microbiology, McGraw Hill(2008).
6. Bayarski, Yuri, "Antibiotics and Their Types, Uses and Side Effects."
7. Bhat M. A, Imran M, Khan S. A, Siddiqui N, Department of pharmaceutical chemistry, Faculty of Pharmacy (Hamdard University) "*Biological activites of sulfonamides"* Indian J. Pharm. Sci., (2005), 67(2): 151-159.
8. Robin R. O, J. Chem. Rev.,1947,38,225.
9. Bhatmal, F. and Parekh H., J. Inst. Chem., 2000, 72, 114.
10. Shah N., Kagathara P., Thakbar V. and Parikh A. R, J. Inst. Chem., 1997, 69, 83.
11. Patel P., Korgaokar S., Parikh K. and Parekh H., Indian J. Chem., 1999, 38b, 696
12. Joshi S. and Khosla N., Bioorg. Med. Chem. Lett., 2003, 13, 3747.
13. Muthukumaran R[1], Sangeetha B, Amutha R, Mathur PP"*Development of anti-HIV activity models of lysine sulfonamide analogs: a QSAR perspective."*Curr Comput Aided Drug Des. *(2012) Mar;8(1):70-82.*
14. Scozzafava A[1], Owa T, Mastrolorenzo A, Supuran CT . "*Anticancer and antiviral sulfonamides."*Curr Med Chem.*(2003) Jun;10(11):925-53.*
15. Selvam P[1], Chandramohan M, De Clercq E, Witvrouw M, Pannecouque C., "*Synthesis and anti-HIV activity of 4-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene) amino]-N(4,6-dimethyl-2-pyrimidinyl)-benzene sulfonamide and its derivatives.",*Eur J Pharm Sci. *(2001) Dec;14(4):313-6.*
16. Mueller J. H. and Hinton J., Proc. Soc. Exp. Biol. Med., (1941), 48:330.

17. Lapage S., Shelton J. and Mitchell T., Methods in Microbiology', Norris J. and Ribbons D., (1970), (Eds.), Vol. 3A, Academic Press, London.
18. MacFaddin J. F., Biochemical Tests for Identification of Medical Bacteria, (2000), 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.
19. Greenberg A.E., Trussell R.R. and Clesceri L.S. (Eds.), Standard Methods for the Examination of Water and Wastewater, (1985), 16th ed., APHA, Washington, D.C.
20. American Public Health Association, Standard Methods for the Examination of Dairy Products,(1978), 14th ed., APHA, Inc., Washington, D.C.
21. American Public Health Association, Standard Methods of Water Analysis,(1917), 3rd ed., APHA, Inc., Washington, D.C.
22. Abbas Q., Department of Microbiolog University of Veterinary and Animal Sciences, Lahore," *Kirby Bauer Antibiotic Sensitivity Test."*Pharmaceutical Microbiology Assignment, (2013).
23. Bag, P., Chattopadhyay, D., Mukherjee, H., Ojha, D., Mondal, N., Chawla Sarkar, M., Das, G. and Chakraborti, S. Anti-herpes virus activities of bioactive fraction and isolated pure constituent of Mallotus peltatus: an ethnomedicine from Andaman Islands.(2012) Virol J 9, 98.
24. Mukherjee, H., Ojha, D., Chandel, H.S., Bhattacharyya, S., Chatterjee, T.K., Mukherjee, P.K., Chakraborti, S. And Chattopadhyay, D. Anti-herpes virus activities of Achyranthes aspera: an Indian ethnomedicine, and its triterpene acid.(2013). Microbiol Res 168, 238–244.
25. Zhang, Y., But, P.H., Ooi, E.C.V., Xu, H.X., Delaney, G.D., Lee Spencer, H.S. and Lee, S.F. Chemical properties, mode of action, and in vivo anti-herpes activities of a lignin carbohydrate complex from Prunella vulgaris. (2007). Antiviral Res 75, 242–249.